Answering the Challenge of Cancer Genetic Testing

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Introduction

Cancer genomes present unique challenges for analytical validation. Diverse variant types (SNVs, SVs, etc), tumor heterogeneity, and archival samples conditions (FFPE) contribute to more complicated genomic analyses in cancer than that of normal germline tissues. Since commercially available standards are of limited utility, we aimed to create a standard for cancer NGS testing to represent the full spectrum of mutations and frequencies. Using both augmented whole exome sequencing and a comprehensive DNA cancer panel (~1500 genes), we determined sensitivity and specificity across a set of gold standard cancer variants.

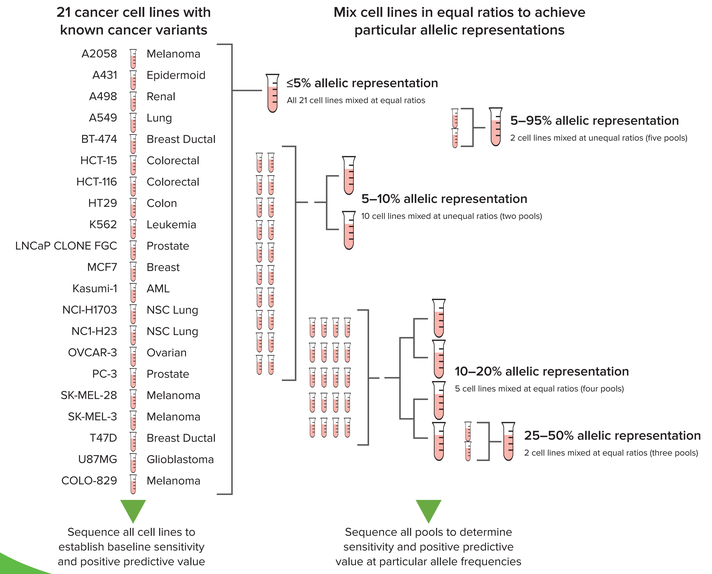
Methods

Cancer Reference Standards

Comprehensive cancer reference standards were created to account for various cancer mutation types and test limits of detection. We procured 38 (DNA cancer panel) and 11 (enhanced whole exome sequencing) well characterized cell lines for analyses.

Limit of Detection for Small Variants

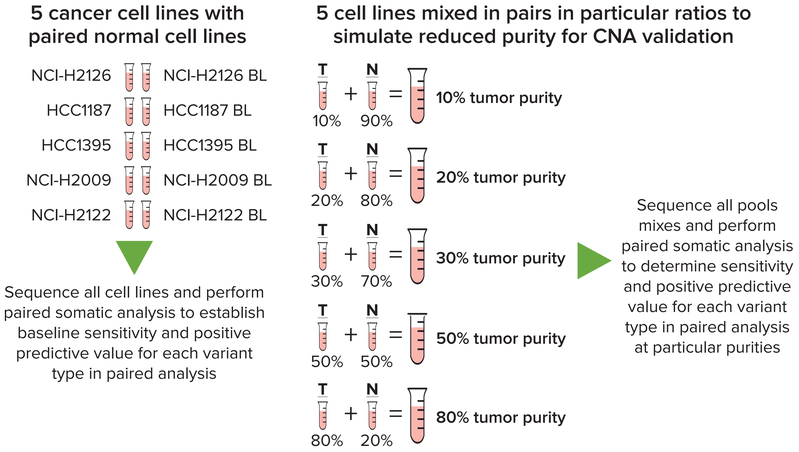
Cell lines were diluted into mixtures to generate minor allele frequencies (MAF) ranging from ≤5% to 95% as shown below for DNA panel testing (a similar approach was used for WES). Dilutions represent ~16,000 SNVs and 675 indels.



Methods — Continued

LOD for Copy Number Alterations

Tumor biopsies are frequently contaminated with surrounding normal tissue. To simulate different levels of normal contamination, cell lines containing CNAs were diluted as shown in the following schema for DNA panel characterization.



Results

Calculating Limits of Detection

Analytical Sensitivity = TP*100/(TP+FN)

LOD Sensitivity Positive Predictive Agreement (PPA) = TP/(TP+FN)

LOD Sensitivity Positive Predictive Value (PPV) = TP/(TP+FP)

Results — Continued

ACE DNA Cancer Panel Performance

Panel Specifications		
Sensitivity	Base Substitutions	≥99% MAF at ≥5%
	Indels	≥99% MAF at ≥10%
	CNAs	96% tumor content ≥30%
Specificity	≥99%*	
Median Depth	≥500x	
Sample Types	FF and FFPE	

* CNAs pending

ACE Cancer Exome Performance for Small Variants

Exome Specifications	LOD at MAF ≥10%	
Variant Type	**Sensitvity (PPA)**	**Specificity (PPV)**
Small Variants	98%	99%
SNPs	98%	99%
Indels	93%	98%
Median Depth	≥200x	
Analytical Mode	Tumor / Normal	

Conclusion

Robust validation of our comprehensive DNA panel and augmented exome demonstrated high sensitivity and specificity. Utilizing this approach, we perform both fresh frozen and formalin treated samples using tumor only and tumor/normal analytical modes with high success rates.

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